On page 10, in the paragraphs of lines 20-35 through page 11, lines 1-2, the text has been amended to read as follows:

B2

-- Figures 1A-1 - 1A-2 show the nucleotide sequence of a native sequence human Apo-2DcR cDNA (SEQ ID NO:2) and its derived amino acid sequence (SEQ ID NO:1) (initiation site assigned at residue 1 (nucleotides 193-195)).

Figures 1B-1 - 1B-2 show the nucleotide sequence of a native sequence human Apo-2DcR cDNA (SEQ ID NO:4) and its derived amino acid sequence (SEQ ID NO:3) (initiation site assigned at residue -40 (nucleotides 73-75)).

Figure 2 shows the primary structure and mRNA expression of Apo-2 and Apo-2DcR. The figure depicts the deduced amino acid sequences of human Apo-2 (SEQ ID NO:11) and Apo-2DcR (SEQ ID NO:1) aligned with full-length DR4 (SEQ ID NO:14). The death domain of Apo-2 is aligned with those of DR4, Apo-3/DR3 (SEQ ID NO:15), TNFR1 (SEQ ID NO:16), and CD95 (SEQ ID NO:17); asterisks indicate residues that are essential for death signaling by TNFR1 [Tartaglia et al., supra]. Indicated are the predicted signal peptide cleavage sites (arrows), the two cysteine-rich domains (CRD1, 2) and the transmembrane domain of Apo-2 and DR4 or the hydrophobic C-terminus of Apo-2DcR (underlined). Also indicated are the five potential N-linked glycosylation sites (black boxes) and the five sequence pseudo-repeats (brackets) of Apo-2DcR. --

On page 11, in the paragraphs of lines 18-24, the text has been amended to read as follows:

B3

-- Figures 8A-1 - 8B show the nucleotide sequence of a native sequence human Apo-2 cDNA and its derived amino acid sequence.

Figure 9 shows the derived amino acid sequence of a native sequence human Apo-2 (SEQ ID NO:11) - the putative signal sequence is underlined, the putative transmembrane domain is boxed, and the putative death domain sequence is dash underlined. The cysteines of the two cysteine-rich domains are individually underlined. --

On page 11, in the paragraphs on lines 33-35 through page 12, lines 1-16, the text has been amended to read as follows:

-- Figures 11A-11E show the induction of apoptosis by Apo-2 and inhibition of Apo-2L activity by soluble Apo-2 ECD. Human 293 cells (A, B) or HeLa cells (C) were transfected by pRK5 vector or by pRK5-based plasmids encoding Apo-2 and/or CrmA. Apoptosis was assessed by morphology (A), DNA fragmentation (B), or by FACS (C-E). Soluble Apo-2L was pre-incubated with buffer or affinity-purified Apo-2 ECD together with anti-Flag antibody or Apo-2 ECD immunoadhesin or DR4 or TNFR1 immunoadhesins and added to HeLa cells. The cells were later analyzed for apoptosis (D). Dose-response analysis using Apo-2L with Apo-2 ECD immunoadhesin was also determined (E).

Figures 12A-12B show activation of NF-κB by Apo-2, DR4, and Apo-2L. (A) HeLa cells were transfected with expression plasmids encoding the indicated proteins. Nuclear extracts were prepared and analyzed by an electrophoretic mobility shift assay. (B) HeLa cells or MCF7 cells were treated with buffer, Apo-2L or TNF-alpha and assayed for NF-κB activity. (C) HeLa cells were preincubated with buffer, ALLN or cyclohexamide before addition of Apo-2L. Apoptosis was later analyzed by FACS. --

On page 12, in the paragraphs on lines 20-31, the text has been amended to read as follows:

-- Figures 14A-14C show the FACS analysis of Apo-2DcR antibodies (illustrated by the bold lines) as compared to IgG controls (dotted lines). The antibodies (4G3.9.9; 6D10.9.7; and 1C5.24.1 respectively) recognized the Apo-2DcR receptor expressed in HUMEC cells.

Figures 15A-15C contain graphs showing results of ELISAs testing binding of Apo-2DcR antibodies 4G3.9.9; 6D10.9.7; and 1C5.24.1 respectively, to Apo-2DcR and to other known Apo-2L receptors referred to as DR4, Apo-2 and DcR2. --

On page 54, in the paragraph on lines 7-23, the text has been amended to read as follows:

-- As described in the Examples below, anti-Apo-2DcR monoclonal antibodies have been prepared. Several of these antibodies, referred to as 4G3.9.9, 6D10.9.7, and 1C5.24.1 have been deposited with ATCC and have been assigned deposit accession numbers HB-12541, HB-12542, and HB-12543, respectively, In one embodiment, the monoclonal antibodies of the invention will have the same biological characteristics as one or more of the antibodies secreted by the hybridoma cell lines deposited under accession numbers HB-12541, HB-12542, or HB-12543. The term "biological characteristics" is used to refer to the *in vitro* and or *in vivo* activities or properties of the monoclonal antibodies, such as the ability to bind to Apo-2DcR or to substantially block, induce, or enhance Apo-2DcR activation. Optionally, the monoclonal antibody will bind to the same epitope as at least one of the three antibodies specifically referred to above. Such epitope binding can be determined by conducting various assays, like those described herein and in the examples. --

On page 72, in the paragraphs on lines 17-24, the text has been amended to read as follows:

-- The cytoplasmic region of Apo-2 contains a death domain (amino acid residues 324-391 shown in Fig. 8 (SEQ ID NO:11); see also Fig. 2) which shows significantly more amino acid sequence identity to the death domain of DR4 (64%) than to the death domain of TNFR1 (30%); CD95 (19%); or Apo-3/DR3 (29%) (Fig. 2). Four out of six death domain amino acids that are required for signaling by TNFR1 [Tartaglia et al., supra] are conserved in Apo-2 while the other two residues are semi-conserved (see Fig. 2). --

On page 73, in the paragraph on lines 14-21, the text has been amended to read as follows:

-- A soluble Apo-2 ECD immunoadhesin construct was prepared. The Apo-2 ECD (amino acids 1-184 shown in Fig. 8 (SEQ ID NO:11)) was fused to the hinge and Fc region of human immunoglobulin G_1 heavy chain in pRK5 as described previously [Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-

B8 Cont'd

10539 (1991)]. The immunoadhesin was expressed by transient transfection into human 293 cells and purified from cell supernatants by protein A affinity chromatography, as described by Ashkenazi et al., supra. --

On page 79, in the paragraph on lines 5-16, the text has been amended to read as follows:

B9

-- Balb/c mice (obtained from Charles River Laboratories) were immunized by injecting 0.5µg/50µl of an Apo-2DcR immunoadhesin protein (diluted in MPL-TDM adjuvant purchased from Ribi Immunochemical Research Inc., Hamilton, MT) 11 times into each hind foot pad at 3 day intervals. The Apo-2DcR immunoadhesin protein was generated by fusing an N-terminal region of Apo-2DcR (amino acids 1-165 shown in Fig. 1A (SEQ ID NO:1)) to the hinge and Fc region of human immunoglobulin G_1 heavy chain in pRK5 as described previously [Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991)]. The immunoadhesin protein was expressed by transient transfection into human 293 cells and purified from cell supernatants by protein A affinity chromatography, as described by Ashkenazi et al., supra. --

On page 83, in the paragraph on lines 3-13, the text has been amended to read as follows:

B10

-- The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia USA (ATCC):

Material	ATCC Dep. No.	Deposit Date
pRK5-Apo-2	209021	May 8, 1997
Apo2-DcR	209087	May 30, 1997
4G3.9.9	HB-12541	June 11, 1998
6D10.9.7	HB-12542	June 11, 1998
1C5.24.1	HB-12543	June 11, 1998

IN THE CLAIMS:

Please cancel without prejudice claims 15-28.

Claims 29-34 have been amended to read as follows:

29. (Amended) The hybridoma cell line deposited as ATCC accession number HB-12541.

30. (Amended) The hybridoma cell line deposited as ATCC accession number HB-12542.

31. (Amended) The hybridoma cell line deposited as ATCC accession number HB-12543.

32. (Amended) The 4G3.9.9 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12541.

33. (Amended) The 6D10.9.7 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12542.

34. (Amended) The 1C5.24.1 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12543.

Please add the following claims:

--55. An isolated antibody which binds to an Apo-2 DcR polypeptide comprising amino acid residues 1 to 259 of Figure 1A (SEQ ID NO:1).

56. An isolated antibody which binds to an extracellular domain of an Apo-2 DcR polypeptide consisting of amino acid residues 1 to X, wherein X is any one of amino acid residues 161 to 236 of Figure 1A (SEQ ID NO:1).

57. The antibody of claim 55 which is a monoclonal antibody.

58. The antibody of claim 56 which is a monoclonal antibody.

59. The antibody of claim 57 which is a chimeric, humanized or human antibody.

60. The antibody of claim 58 which is a chimeric, humanized or human antibody.

B17 cont'd

61. The antibody of claim 57 which is a blocking antibody.

62. The antibody of claim 58 which is a blocking antibody.

63. The antibody of claim 55 or 56 which, in addition to binding said Apo-2 DcR polypeptide, binds to another Apo-2 ligand receptor selected from the group consisting of DR4, Apo-2, DcR2, and OPG.

64. The antibody of claim 55 or 56 which binds to the same epitope to which the 4G3.9.9 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12541 binds.

65. The antibody of claim 55 or 56 which binds to the same epitope to which the 6D10.9.7 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12542 binds.

66. The antibody of claim 55 or 56 which binds to the same epitope to which the 1C5.24.1 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number HB-12543 binds.

67. The antibody of claim 61 which blocks binding of Apo-2 ligand to said Apo-2 DcR polypeptide.

68. The antibody of claim 62 which blocks binding of Apo-2 ligand to said Apo-2 DcR polypeptide. --

REMARKS

In Applicant's Continuation Application Transmittal filed June 21, 2001, claims 1-14 and 35-54 were canceled without prejudice. Accordingly, claims 15-34 are currently pending in the application. In the above amendment, claims 15-28 have been canceled without prejudice. In the amendment, claims 29-34 and the specification have been amended to recite the accession numbers assigned by the ATCC for the deposited material referred to in Applicant's specification. Claims 55-68 have been added. Support for the added claims can be found on at least pages 10, 13, 14, 16-18, 50-56, and 79-81 of the specification, and thus do not introduce new matter. The amendments are illustrated on the attached pages entitled "Marked Up Version to Show Changes Made". For the Examiner's convenience, a clean copy of the now pending claims 29-34 and 55-68 are provided above.

A Notice to File Corrected Application Papers was mailed on August 9, 2001. Each of the items referred to in that Notice are discussed below in Sections "A" and "B".

A. Sequence Listing

A substitute paper copy of the Sequence Listing for the present application is being filed herewith. Applicant wishes to advise that the Sequence Listing previously filed in the application did not include several of the sequences provided in Figure 2. Therefore, Applicant is filing herewith a substitute paper copy which properly includes the sequences referred to in Figure 2. These sequences now included in the substitute Sequence Listing are assigned SEQ ID NO:s 14-17, and are found in Figure 2 as originally filed in the application. This substitute Sequence Listing is believed to be in full compliance with the Sequence Listing rules provided in 37 CFR Section 1.821.

A computer readable form of this substitute Sequence Listing was filed in Applicant's prior application. Accordingly, Applicant is filing herewith a Request to Use the electronic form of the sequence listing filed in the prior application for the instant application. A Certificate Regarding the Sequence Listing is also enclosed herewith.

The specification, including the Brief Description of the Drawings,

has been amended to recite the respective assigned Sequence Listing identifiers for the sequences referenced in the text and drawings.

B. Drawings

Applicant is filing herewith a substitute set of drawings for the application. It is believed that the substitute drawings are in full compliance with the requirements of 37 CFR 1.84. The Brief Description of the Drawings on pages 10-12 of the specification has been amended, as shown herein, to accurately reflect the figure numbers that appear on the substitute drawings.

C. Nomenclature

Applicant is aware that terms used to refer to polypeptides related to Apo-2DcR include "TRAIL-BP", "hApo-9" and "TNFR-5". These various terms have been employed, for instance, in the published patent application documents cited in Applicant's Information Disclosure Statement.

Respectfully submitted,

GENENTECH, INC.

Date: September 20, 2001

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MARKED UP VERSION TO SHOW CHANGES MADE

In the specification:

On page 1, lines 10-13, the text has been amended as follows:

-- This is a continuation application of application serial no. 09/096,500 filed June 12, 1998, which is a non-provisional application claiming priority under Section 119(e) to provisional application number 60/049,911 filed June 18, 1997, the contents of which are incorporated herein by reference. --

On page 10, in the paragraphs of lines 20-35 through page 11, lines 1-2, the text has been amended as follows:

-- Figures 1A-1 - 1A-2 show[s] the nucleotide sequence of a native sequence human Apo-2DcR cDNA (SEQ ID NO:2) and its derived amino acid sequence (SEQ ID NO:1) (initiation site assigned at residue 1 (nucleotides 193-195)).

Figures 1B-1 - 1B-2 show[s] the nucleotide sequence of a native sequence human Apo-2DcR cDNA (SEQ ID NO:4) and its derived amino acid sequence (SEQ ID NO:3) (initiation site assigned at residue -40 (nucleotides 73-75)).

Figure 2 shows the primary structure and mRNA expression of Apo-2 and Apo-2DcR. The figure depicts the deduced amino acid sequences of human Apo-2 (SEQ ID NO:11) and Apo-2DcR (SEQ ID NO:1) aligned with full-length DR4 (SEQ ID NO:14). The death domain of Apo-2 is aligned with those of DR4, Apo-3/DR3 (SEQ ID NO:15), TNFR1 (SEQ ID NO:16), and CD95 (SEQ ID NO:17); asterisks indicate residues that are essential for death signaling by TNFR1 [Tartaglia et al., supra]. Indicated are the predicted signal peptide cleavage sites (arrows), the two cysteine-rich domains (CRD1, 2) and the transmembrane domain of Apo-2 and DR4 or the hydrophobic C-terminus of Apo-2DcR (underlined). Also indicated are the five potential N-linked glycosylation sites (black boxes) and the five sequence pseudo-repeats (brackets) of Apo-2DcR. --

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-- Figures 8A-1 - 8B show[s] the nucleotide sequence of a native sequence human Apo-2 cDNA and its derived amino acid sequence.

Figure 9 shows the derived amino acid sequence of a native sequence human Apo-2 (SEQ ID NO:11) - the putative signal sequence is underlined, the putative transmembrane domain is boxed, and the putative death domain sequence is dash underlined. The cysteines of the two cysteine-rich domains are individually underlined. --

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-- Figures 11A-11E show[s] the induction of apoptosis by Apo-2 and

inhibition of Apo-2L activity by soluble Apo-2 ECD. Human 293 cells (A, B) or HeLa cells (C) were transfected by pRK5 vector or by pRK5-based plasmids encoding Apo-2 and/or CrmA. Apoptosis was assessed by morphology (A), DNA fragmentation (B), or by FACS (C-E). Soluble Apo-2L was pre-incubated with buffer or affinity-purified Apo-2 ECD together with anti-Flag antibody or Apo-2 ECD immunoadhesin or DR4 or TNFR1 immunoadhesins and added to HeLa cells. The cells were later analyzed for apoptosis (D). Dose-response analysis using Apo-2L with Apo-2 ECD immunoadhesin was also determined (E).

Figures 12A-12B show[s] activation of NF-κB by Apo-2, DR4, and Apo-2L. (A) HeLa cells were transfected with expression plasmids encoding the indicated proteins. Nuclear extracts were prepared and analyzed by an electrophoretic mobility shift assay. (B) HeLa cells or MCF7 cells were treated with buffer, Apo-2L or TNF-alpha and assayed for NF-κB activity. (C) HeLa cells were preincubated with buffer, ALLN or cyclohexamide before addition of Apo-2L. Apoptosis was later analyzed by FACS. --

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Figures 15A-15C contain[s] graphs showing results of ELISAs testing binding of Apo-2DcR antibodies 4G3.9.9; 6D10.9.7; and 1C5.24.1 respectively, to Apo-2DcR and to other known Apo-2L receptors referred to as DR4, Apo-2 and DcR2. --

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On page 79, in the paragraph on lines 5-16, the text has been amended as follows:

-- Balb/c mice (obtained from Charles River Laboratories) were immunized by injecting 0.5µg/50µl of an Apo-2DcR immunoadhesin protein (diluted in MPL-TDM adjuvant purchased from Ribi Immunochemical Research Inc., Hamilton, MT) 11 times into each hind foot pad at 3 day intervals. The Apo-2DcR immunoadhesin protein was generated by fusing an N-terminal region of Apo-2DcR (amino acids 1-165 shown in Fig. 1A (SEQ ID NO:1)) to the hinge and Fc region of human immunoglobulin G_1 heavy chain in pRK5 as described previously [Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991)]. The immunoadhesin protein was expressed by transient transfection into human 293 cells and purified from cell supernatants by protein A affinity chromatography, as described by Ashkenazi et al., supra. --

On page 83, in the paragraph on lines 3-13, the text has been amended as follows:

-- The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia USA (ATCC):

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Apo2-DcR	209087	May 30, 1997
4G3.9.9	HB-12541	June 11, 1998
6D10.9.7	HB-12542	June 11, 1998
1C5.24.1	HB-12543	June 11, 1998